International Journal of Diabetes Mellitus 2 (2010) 141–143

Contents lists available at ScienceDirect

International Journal of Diabetes Mellitus

journal homepage: www.elsevier.com/locate/ijdm

Original Article

Free radical activity in hypertensive type 2 diabetic patients

Suvarna Prasad *, Ajay Kumar Sinha

Department of Biochemistry, M. M. Institute of Medical Sciences & Reasearch, Mullana, Ambala, Haryana, India

ARTICLE INFO

Article history:
Received 24 July 2010
Accepted 4 October 2010

Keywords:
Superoxide dismutase
Nitric oxide
Malondialdehyde
Type 2 diabetes mellitus
Hypertension

ABSTRACT

Background: Free radical activity is an important cause of vascular complications in type 1 diabetes mellitus. But data regarding vascular complications in type 2 diabetes mellitus are scant.
Objectives: The aim of this study was to examine free radical activity in type 2 diabetic patients with hypertension compared to those without hypertension.
Materials and Methods: The serum levels of lipid peroxidation product, MDA malondialdehyde), the free radical scavenger, SOD (superoxide dismutase) and NO (nitric oxide) were studied in 50 type 2 diabetic outpatients. Controls were regarded as those diabetic outpatients who did not have hypertension.
Result: Among 50 patients thus studied 19 were hypertensive. The concentration (median (range)) of both SOD (21.31(5.33–26.64) vs. 16.65(6.66–22.64) U/dl; $p < 0.05$) and NO (18.54 (11.40–37.07) vs. 21.39(15.69–35.65) U/dl; $p < 0.05$) were reduced in the hypertensive group. Similarly, concentration (median (range) of MDA (359(231–718) vs. 385(256–666) μmoles/dl; $p < 0.01$ were increased in the hypertensive group.
Conclusion: The reduction in serum levels of SOD and NO with a concomitant increase in serum MDA levels is consistent with an increase in free radical activity in hypertensive type 2 diabetics.

1. Introduction

Free radical activity has been implicated in the development of diabetic vascular complications in type 1 diabetes mellitus. It plays an important role in both microvascular and macrovascular complications in diabetes mellitus. However, data regarding vascular complications in type 2 diabetes mellitus are scant. Cardiovascular diseases (CVD) are the major causes of mortality in persons with diabetes, and many factors including hypertension contribute to this high prevalence of CVD. Hypertension is twice as frequent in patients with diabetes compared with patients without the disease. Furthermore, up to 75% of CVD in diabetes may be attributable to hypertension, leading to recommendations for more aggressive treatment for those having hypertension in this disease [1].

Long-term complications of diabetes are supposed to be, at least in part, mediated by increased free radical generation and subsequent oxidative stress. In this study, we have attempted to summarize the experimental evidence in this field, and to emphasize the possible importance of oxidative stress in the development of diabetic vascular complications.

Free radicals may be defined as any chemical species that contains unpaired electrons. Unpaired electrons increase the chemical reactivity of an atom or a molecule. Common examples of free radicals include the hydroxyl radical (.0H), super oxide anion (0_2^-), transition metals, such as iron (Fe), copper (Cu), nitric oxide (NO) and peroxynitrite (.OONO) [2]. Free radicals and reactive nonradical species derived from radicals exist in biological cells and tissues at very low concentrations [3,4]. Halliwell and Gutteridge [3] have defined antioxidants as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates, and thus, to significantly delay or inhibit the oxidation of these substrates. This definition includes the enzymes SOD, glutathione peroxidase (GPx) and catalase, as well as nonenzymatic compounds such as tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), and glutathione, which scavenge the reactive oxygen species.

2. Materials and methods

The aim of this study was to investigate whether the serum levels of lipid peroxidation product, malondialdehyde (MDA), serum superoxide dismutase (SOD) and serum nitric oxide (NO) were altered in normotensive type 2 diabetic patients and type 2 diabetic patients who subsequently developed hypertension.

We selected 50 type 2 diabetic patients. 19 of these type 2 diabetic patients had subsequently developed hypertension. The criteria for hypertension were a mean arterial pressure of greater than the upper range of accepted normal pressure, and a mean arterial pressure of greater than 110 mm of Hg (normal is 90 mm of Hg) that is considered to be hypertensive.

* Corresponding author. Address: House No. E-54, GH-94, SEC-20, Panchkula, Haryana 134112, India. Tel.: +91 9872174466, +91 9257206509.
E-mail address: Suvarnaprasad1@Rediffmail.com (S. Prasad).

1877-5934 © 2010 *International Journal of Diabetes Mellitus*. Published by Elsevier Ltd. Open access under CC BY-NC-ND license.
doi:10.1016/j.ijdm.2010.10.002

Type 2 diabetic patients were subjected to an evaluation of their hypertensive state by measuring their blood pressure, three times a day for a period of one week, and the average was taken for the evaluation of blood (mean arterial blood pressure = 1/3 of pulse pressure + diastolic pressure). Blood pressure was measured in the supine position, manually in both arms, by a calibrated sphygmomanometer.

Subjects underwent a medical history screening, a physical examination and laboratory analysis, which included CBC, serum electrolytes, blood urea, creatinine, fasting blood glucose and HbAc 1, ECG, and echocardiography.

Exclusion criteria included tobacco, caffeine use, cardiac and pulmonary disease and evidence of left ventricular hypertrophy. The hypertensive patients were on calcium channel blockers. All medications were stopped 12 h before blood sample collection.

In the morning fasting blood sample was taken. Venous blood was collected from the anterior aspect of the forearm with the help of disposable syringes. Serum was separated within 1 h after refrigeration. The straw colored supernatant serum was centrifuged and separated. All three tests were carried out within 2 h, after obtaining venous blood.

The method of testing for MDA (malondialdehyde) as a marker of lipid peroxidation product was that of determined through the method of Okhawa et al. (1974) who measured MDA as thiobarbituric acid reactive substances (TBARS) [5]. Superoxide dismutase was assessed by the method of Kakkar et al. [6]. Nitric oxide was assessed through the method of Green et al. [7]. In this method, nitric oxide in serum was estimated indirectly by measuring the amount of nitrates formed from nitric oxide.

3. Observation and results

These results are consistent with a significant increase in free radical activity in type 2 diabetic patients with coexistent hypertension. The SOD and NO levels were significantly decreased and MDA levels were significantly increased in those type 2 diabetic patients who had coexistent hypertension (Table 1).

4. Discussion

Increased concentrations of oxygen-derived free radicals are implicated in the pathogenesis of vascular complications in diabetes. Superoxide anion appears to block endothelium derived nitric oxide mediated relaxation by inactivating the eNOS. In a hyperglycemic state, the production of superoxide is stimulated, and the enzyme superoxide dismutase is inhibited by non-enzymatic glycosylation known as Maillard reaction [8]. Glycation was shown to affect the C-terminal end of the enzyme, reducing its heparin binding affinity. Thus, protection against extra cellular radicals by cell surface attached SOD may be impaired in diabetes leaving the endothelial cell susceptible to damage by super oxide anion. The addition of exogenous SOD restores normal or unmasks even greater acetylcholine induced relaxation in diabetic aorta. Thus, in diabetic conditions, normal levels of antioxidant enzymes may be insufficient or may be functionally impaired, so as to preserve a physiological contractile response [9].

Nitric oxide and superoxide anion readily react to form peroxynitrite (OONO) at nearly diffusion limited rate. During physiologic conditions O_2^- scavengers and formation of $OONO^-$ are minimal. During pathologic conditions such as in the presence of increased concentrations of O_2^- or after O_2^- scavengers are exhausted, significant concentrations of $OONO^-$ may be produced. Peroxynitrite directly causes oxidation, peroxidation, and nitration of biologically important molecules (e.g. lipids protein and DNA). It is more cytotoxic than NO in a variety of experimental conditions [10].

An important example of a reaction caused by $OONO^-$ is the nitration of tyrosine. Tyrosine nitration inhibits tyrosine phosphorylation, alters the dynamics of assembly and disassembly of cytoskeletal proteins, and inhibits tyrosine hydroxylase, thereby inhibiting the cycloskeletal movements of endothelial cells [10].

Nitric oxide has contrasting effects on lipids, particularly on oxidation of LDL lipoproteins in the pathogenesis of atherosclerotic lesions. NO inhibits lipid peroxidation by inhibiting radical chain propagation, reactions via radical reaction with lipid peroxyl and alkoxyl group. As a ligand to iron (and other transition metals), NO modulates the peroxidant effects of iron and thereby limits the formation of hydroxyl radicals and iron dependent electron transfer reactions. NO inhibits all and $OONO^-$ mediated lipoprotein oxidation in macrophage and endothelial cell systems. However, NO, induced $OONO^-$ formation can oxidize low density lipoproteins to potentially atherogenic species. In summary, $OONO^-$ is more cytotoxic than NO in a variety of experimental systems and the balance of NO, O_2^-, and $OONO^-$, scavenging systems determine whether biologically relevant $OONO^-$ concentrations will occur in tissues. Thus, the endothelium appears to modulate vascular functions by releasing relaxant substances like NO and constrictor substances like superoxide. Superoxide may play a key role in the relationship between cardiovascular diseases and metabolic disorders like diabetes mellitus, and will almost certainly prove to be a focus for future therapies [10].

Table 1
Clinical and Laboratory data of diabetic patients without hypertension as control/diabetic patients with hypertension.

Parameters	Controls	Hypertensives
Number	31	19
Sex (M/F)	24/7	15/4
Age (In years)	54 (35–65)	51 (40–71)
Wt. (kg)	54 (40–70)	52 (40–71)
Duration of diabetes (years)	4 (0^A–14)	3 (0–20)
Fasting plasma glucose (mg/dl)	175 ± 67	223± 84 $p < 0.001$
Glycosylated Haemoglobin (%)	9.6 ± 1.7	11 ± 2.8 $p < 0.001$
Mean arterial pressure (mmHg)	97 ± 2	116 ± 6 $p < 0.05$
MDA (moles/dl)	359 (231–718) $p < 0.01$, SD ± 55.85	385 (256–666), SD ± 63.34
SOD U^B/dl	21.31 (5.33–26.64) SD ± 4.64	16.65 (6.66–22.64) SD ± 4.08 $p < 0.05$
NO (U/dl)	18.54 (11.40–37.07) SD + 4.34	21.39 (15.69–35.65) SD ± 4.79 $p < 0.05$,

Median (Range), SOD, superoxide dismutase, NO, nitric oxide; MDA, malondialdehdye.
A, newly diagnosed type 2 diabetic patients.
B, one unit of enzyme is defined as enzyme concentration required to inhibit the optical density of chromogen production by 50% in 1 min.
Table 1 shows the clinical and biochemical details of the study groups. There were no significant differences in the age, weight, sex, and duration of diabetes between the two study groups. Patients with hypertension had higher plasma fasting glucose levels ($p < 0.001$) and glycosylated hemoglobin levels ($p < 0.001$) compared to those without hypertension.

5. Conclusion

Much evidence suggests that free radical over generation may be considered the key in the generation of insulin resistance, diabetes and cardiovascular disease. Many new specific causal antioxidants are being developed [10,11], and may become important tools in opposing the increasing epidemic of diabetes a real emergency in our future. It has been demonstrated that insulin resistance is associated in humans with reduced intracellular antioxidant defense [12], and that diabetic subjects prone to complications may have a defective intracellular antioxidant response [13,14] where what we call genetic predisposition to diabetes, as well as liability to its late complications, might be based on a deficient ROS-scavenging ability in β-cells and/or in target tissues such as endothelium.

Oxidative stress is involved in various cardiovascular diseases, including atherosclerosis, hypertension and the aging process; therefore, therapeutic strategies to modulate this maladaptive response should become a target for future extensive investigation, and could have a broad application [15].

References

[1] Sowers James R, Murray E, Edward DF. Diabetes, hypertension, and cardiovascular disease: an update. Hypertension 2001;37:1053–9.
[2] Betteridge DJ. What is oxidative stress? Metabolism 2000;49:3–8.
[3] Tirzitis G, Bartosz G. Determination of antiradical and antioxidant activity: basic principles and new insights. Acta Biochim Pol 2010;57:139–42.
[4] Sies H. Strategies of antioxidant defence. Eur J Biochem 2005;215:213–9.
[5] Ohkawa H, Ohishi N, Yogi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Annal Biochem 1979;95:351–8.
[6] Kakkar P, Awasthi S, Vishwanathan PN. Oxidative changes in brain of aniline exposed rats. Arch Environ Contam Toxicol 1992;23:307–9.
[7] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannebaum SR. Analysis of nitrate, nitrite, and [N 15] nitrate in biological fluids. Anal Biochem 1982;126:131–8.
[8] Taboshashi K, Saito Y. The role of superoxide anion in relationship between the cardiovascular diseases and the metabolic disorders associated with obesity. Nippon Rinsho 2000;58:1592–7.
[9] Szaleczky E, Prechl J, Feher J, Somogyi A. Alterations in enzymatic defence in diabetes mellitus – a rational approach. Postgrad Med J 1999;75:13–7.
[10] Ceriello A. New Insights on oxidative stress and diabetic complications may lead to a causal antioxidant therapy. Diabetes Care 2003;26:1589–96.
[11] Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 2001;53:1159.
[12] Ceriello A, Morocutti A, Mercuri F, Quagliaro L, Moro M, Damante G, et al. Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. Diabetes 2000;49:2170–7.
[13] Hodgkinson AD, Bartlett T, Oates PJ, Millward BA, Demaine AG. The response of antioxidant genes to hyperglycaemia is abnormal in patients with type 1 diabetes and diabetic nephropathy. Diabetes 2003;52:846–51.
[14] Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA is reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defence mechanism. Diabetes 2003;52:2338–45.
[15] Tsutsui Hiroyuki, Kinugawa Shintaro, Matsushima Shouji. Mitochondrial oxidative stress and dysfunction in myocardial remodelling. Cardiovasc Res 2009;81:449–56.